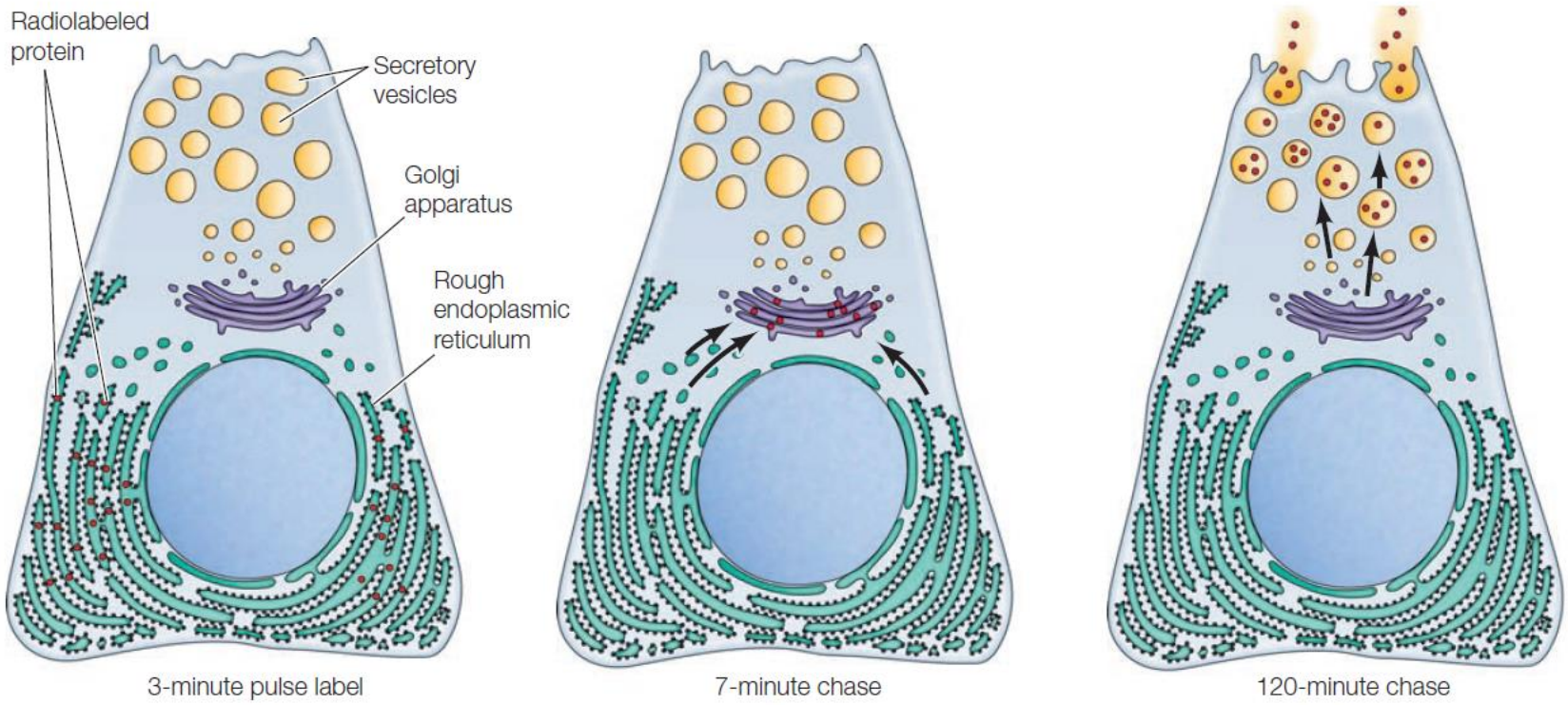
Protein Sorting and Transport: Endoplasmic reticulum

By
Dr. Jyotsnarani Pradhan
Assistant Professor
Department of Biotechnology
Utkal University

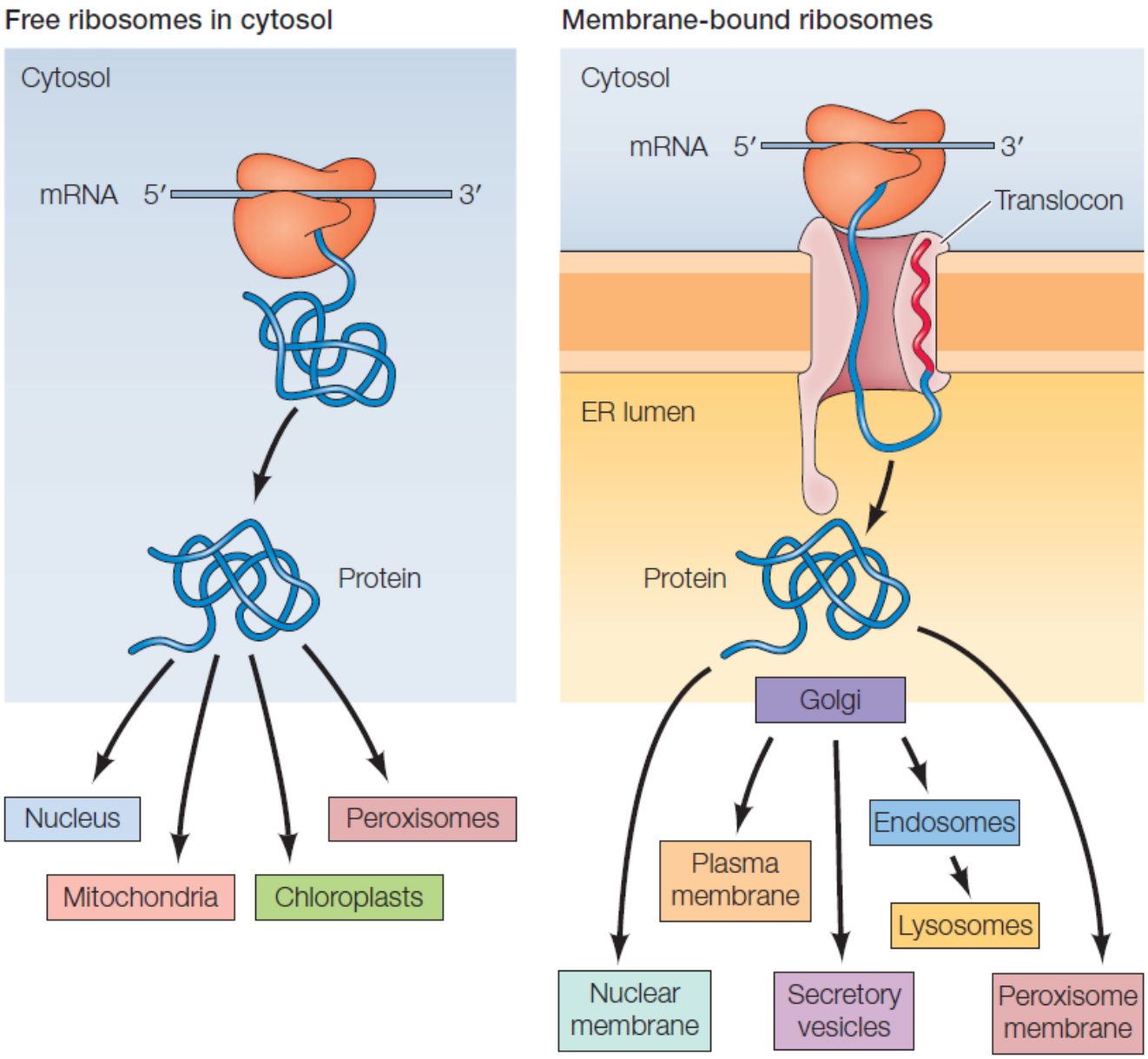
The secretory pathway



Pancreatic acinar cells, which secrete most of their newly synthesized proteins into the digestive tract, were labeled with radioactive amino acids to study the intracellular pathway taken by secreted proteins. After a 3-minute incubation with radioactive amino acids (a "pulse"), autoradiography revealed that newly synthesized proteins were localized to the rough ER. Following further incubation with nonradioactive amino acids (a "chase"), proteins were found to move from the ER to the Golgi apparatus and then, within secretory vesicles, to the plasma membrane and cell exterior.

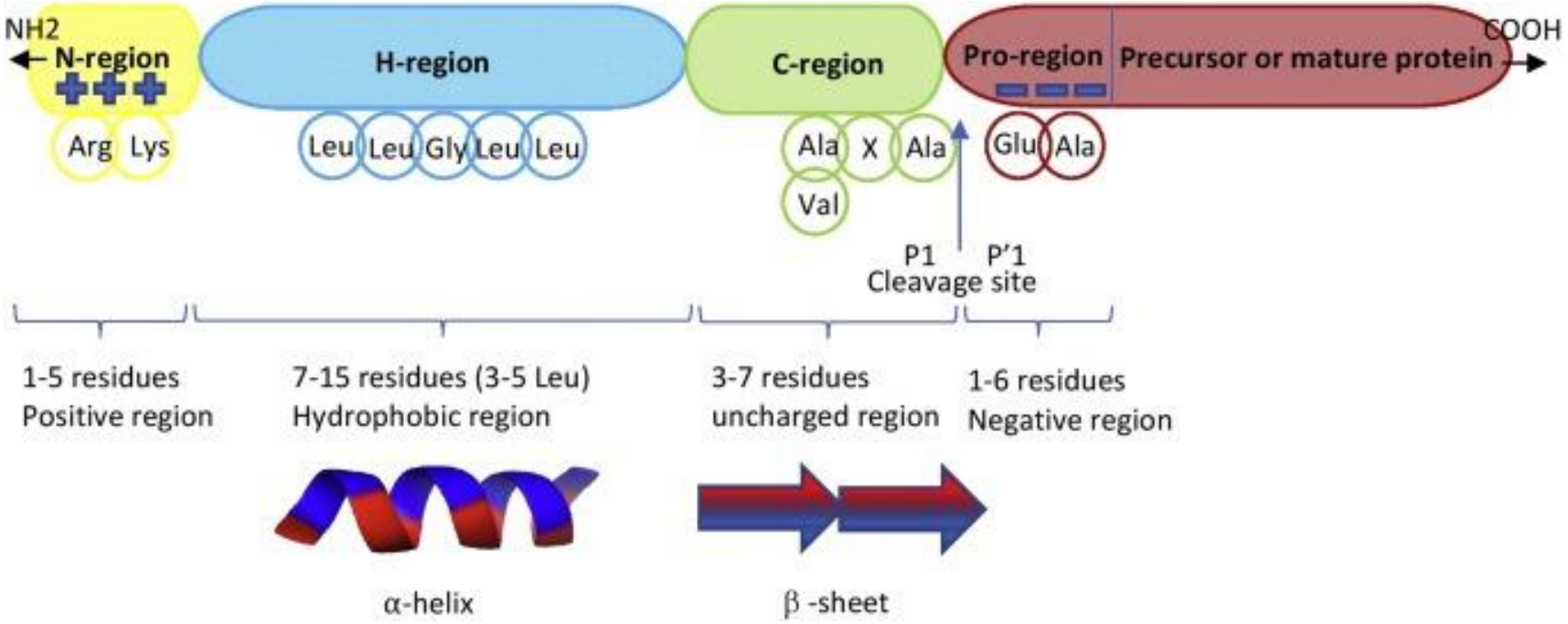
The Cell: A Molecular Approach by G.M. Cooper, 8th Ed.

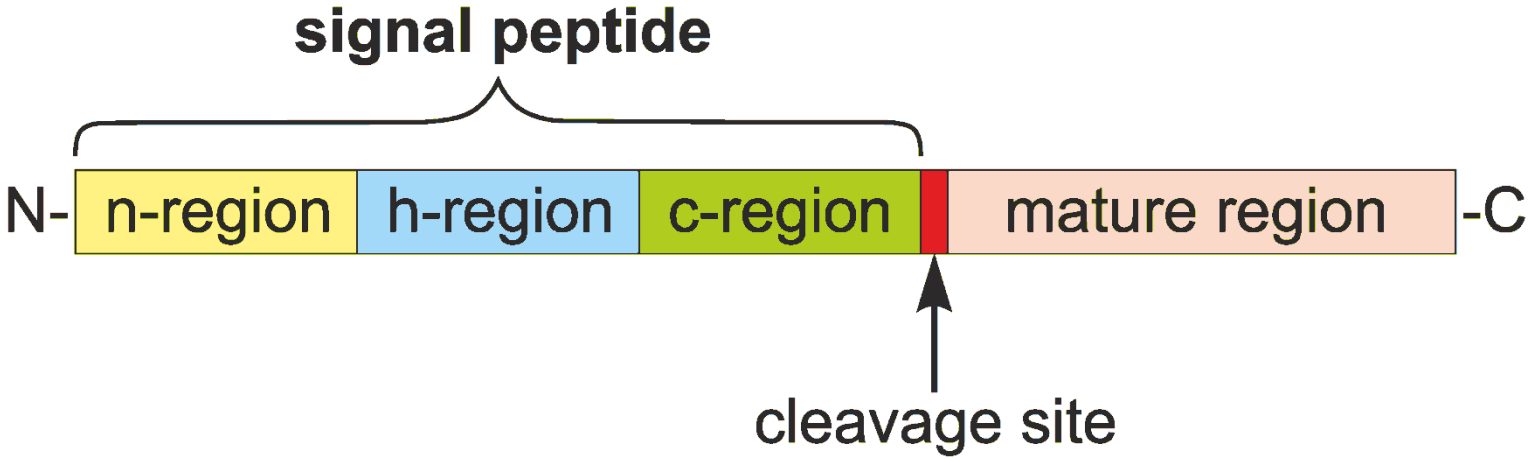
Overview of protein sorting



In higher eukaryotic cells, the initial sorting of proteins to the ER takes place while translation is in progress. Proteins synthesized on free ribosomes either remain in the cytosol or are transported to the nucleus, mitochondria, chloroplasts, or peroxisomes. In contrast, proteins synthesized on membrane-bound ribosomes are translocated directly into the ER through the translocon. These proteins contain signal sequences (indicated in red) that are cleaved during translocation. Proteins that are translocated into the ER may be either retained within the ER or transported to nuclear membranes, peroxisomal membranes, or the Golgi apparatus and, from there, to endosomes, lysosomes, the plasma membrane, or the cell exterior via secretory vesicles.

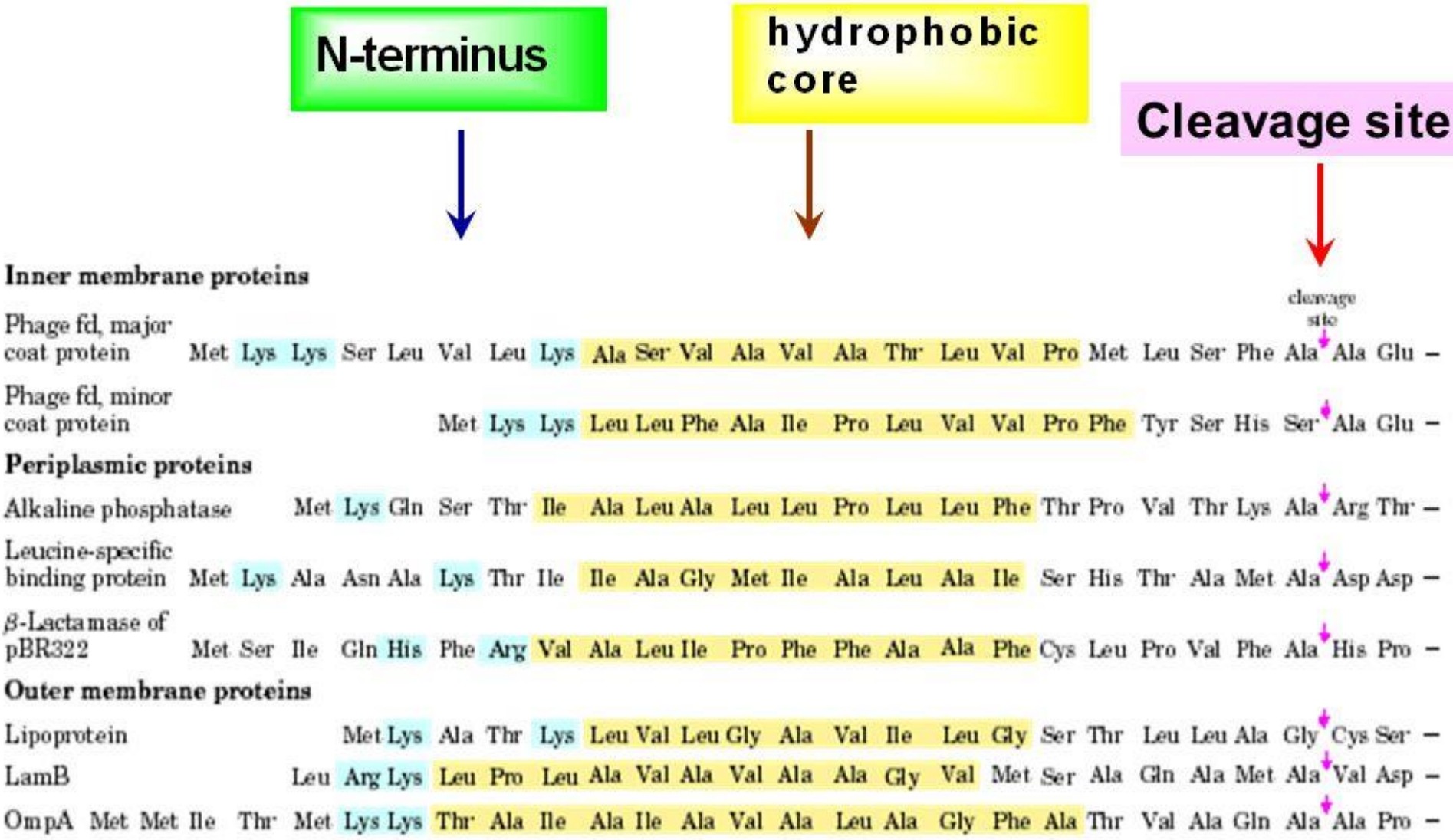
The Cell: A Molecular Approach by G.M. Cooper, 8th Ed.



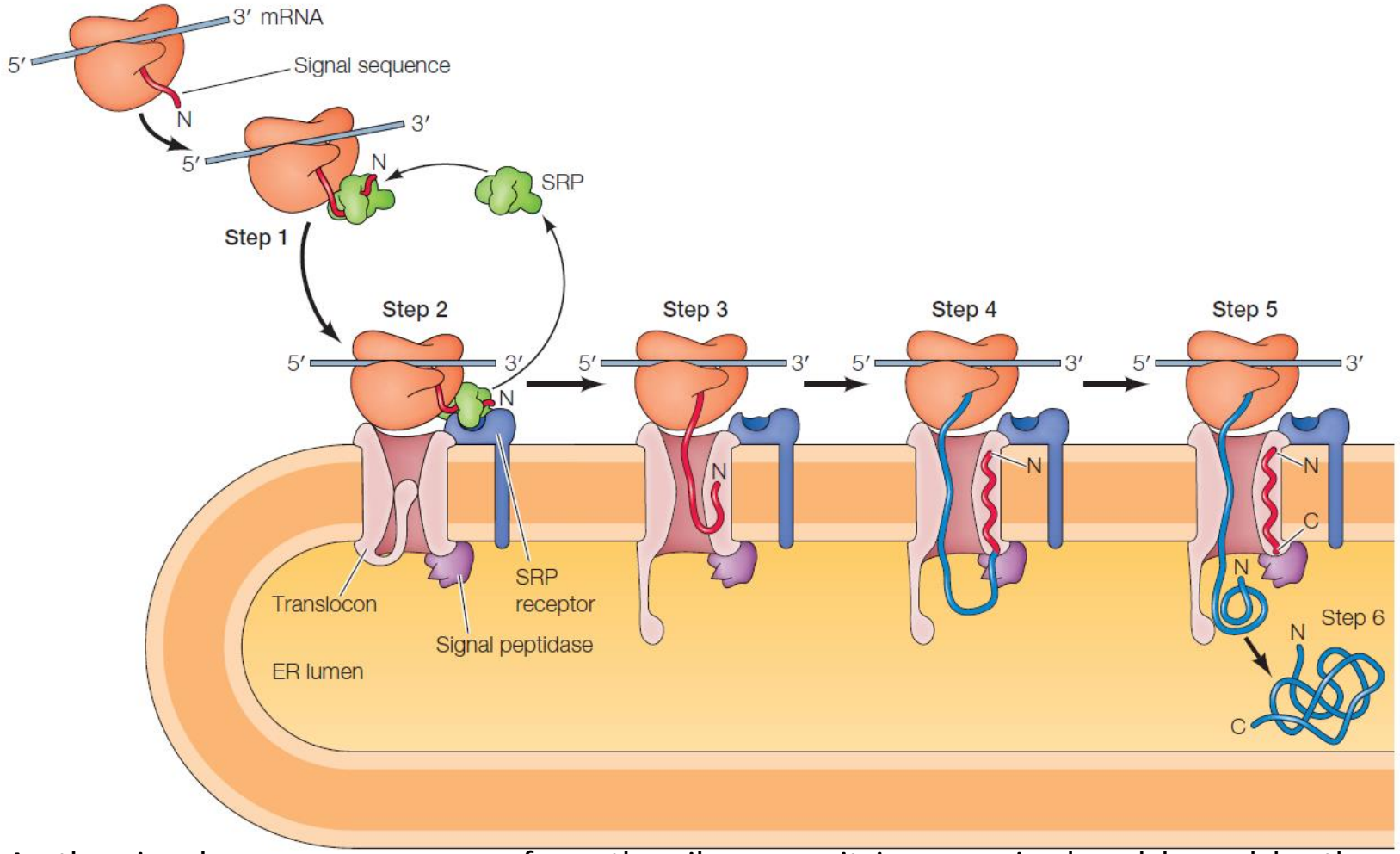


The signal sequence of growth hormone

Signal sequence for ER



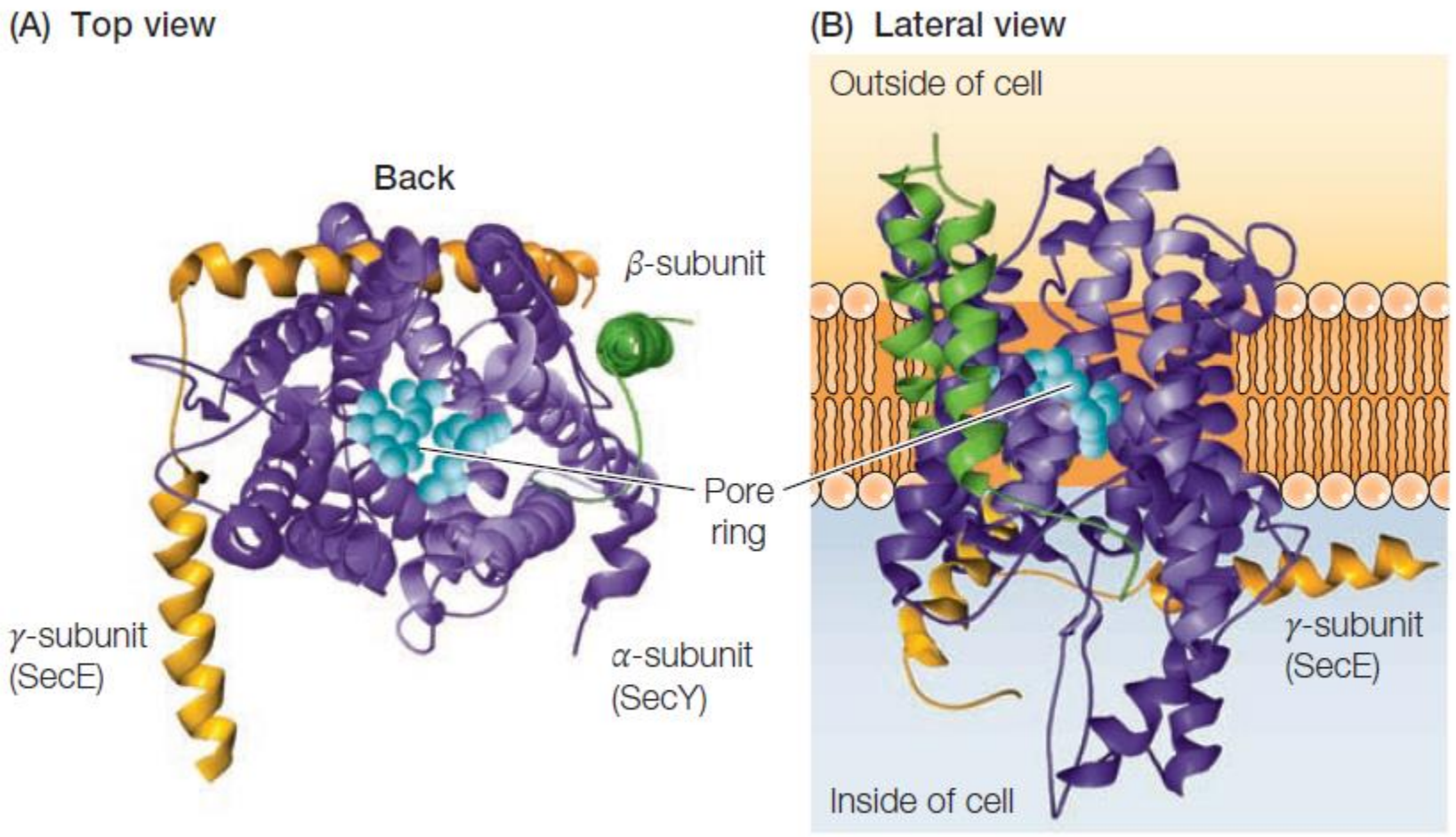
Cotranslational targeting of secretory proteins to the ER



Step 1: As the signal sequence emerges from the ribosome, it is recognized and bound by the signal recognition particle (SRP). Step 2: The SRP escorts the complex to the ER membrane where it binds to the SRP receptor. Step 3: The SRP is released, the ribosome binds to the translocon, and insertion of the signal sequence opens the translocon. Step 4: Translation resumes and the signal sequence is cleaved by signal peptidase. Step 5: Continued translation drives translocation of the growing polypeptide chain across the membrane. Step 6: The completed polypeptide chain is released within the ER lumen

The Cell: A Molecular Approach by G.M. Cooper, 8th Ed.

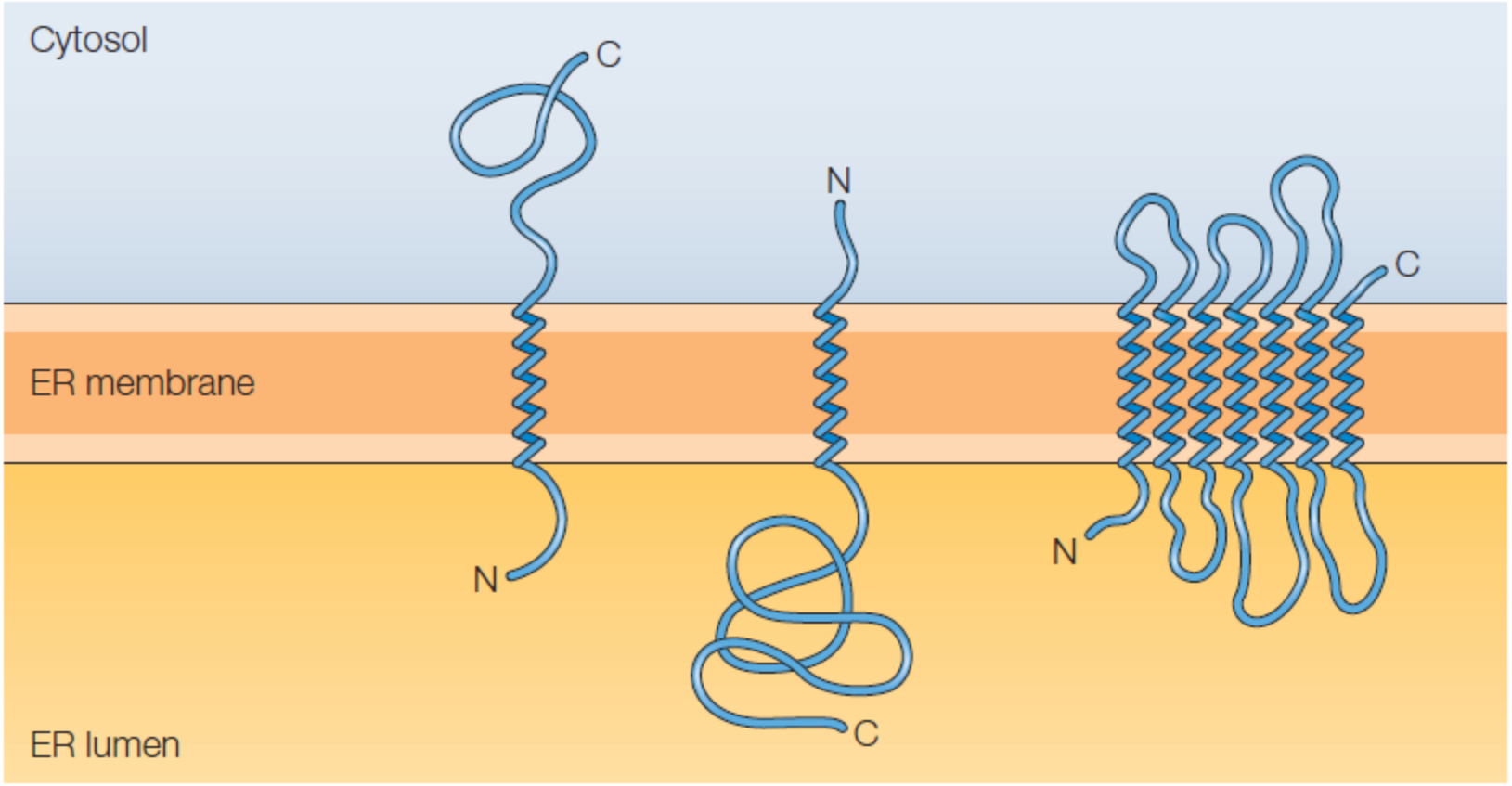
Structure of the translocon



The translocon consists of three transmembrane subunits, shown in green, purple, and yellow. (A) Top view from the cytosol, showing the plug in the translocon channel. (B) Lateral view of the translocon inserted into the ER membrane. (After E. Park and T. A. Rapoport, 2012. Ann. Rev. Biophys. 41: 21.)

The Cell: A Molecular Approach by G.M. Cooper, 8th Ed.

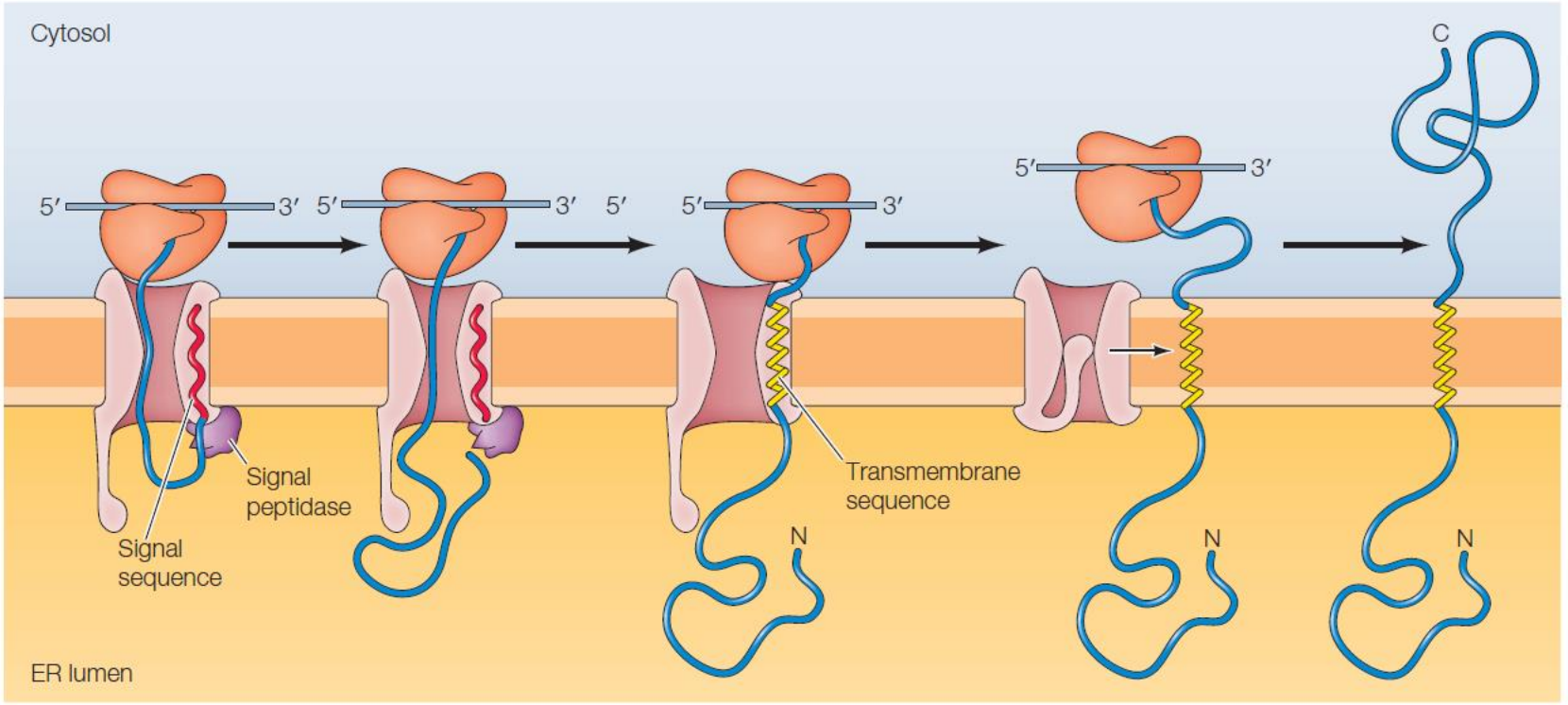
Orientations of membrane proteins



proteins span the membrane via α-helical regions of 20–25 hydrophobic amino acids, which can be inserted in a variety of orientations. The proteins at left and center each span the membrane once, but they differ in whether the carboxy (C) or amino (N) terminus is on the cytosolic side. On the right is an example of a protein that has multiple membrane-spanning regions.

The Cell: A Molecular Approach by G.M. Cooper, 8th Ed.

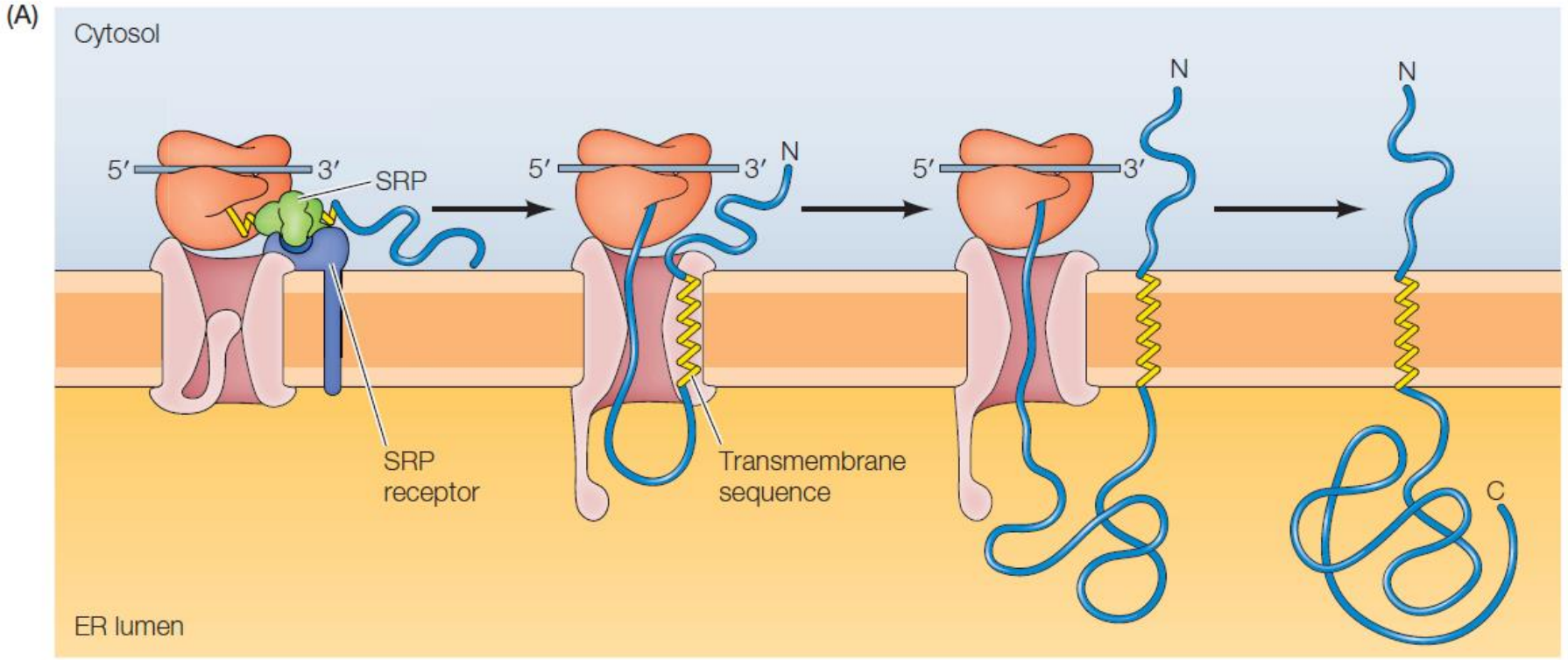
Insertion of a membrane protein with a cleavable signal sequence



The signal sequence is cleaved as the polypeptide chain crosses the membrane, so the amino (N) terminus of the polypeptide chain is exposed in the ER lumen. However, translocation of the polypeptide chain across the membrane is halted when the translocon recognizes a transmembrane sequence. This allows the protein to exit the translocon laterally and become anchored in the ER membrane. Continued translation results in a membrane-spanning protein with its carboxy (C) terminus on the cytosolic side.

The Cell: A Molecular Approach by G.M. Cooper, 8th Ed.

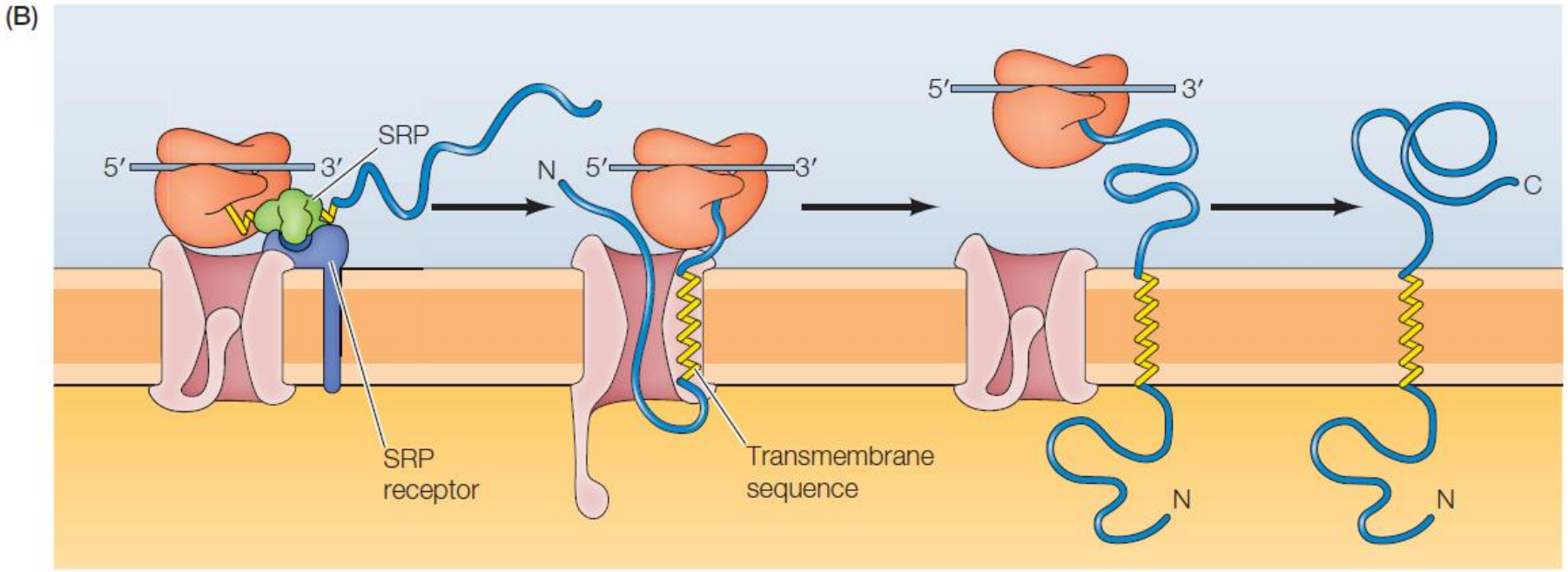
Insertion of membrane proteins via internal transmembrane sequences



Internal transmembrane sequences can lead to the insertion of polypeptide chains in either orientation in the ER membrane. (A) The transmembrane sequence directs insertion of the polypeptide such that its amino (N) terminus is exposed on the cytosolic side. The transmembrane sequence exits the translocon to anchor the protein in the lipid bilayer and the remainder of the polypeptide chain is translocated into the ER as translation proceeds.

The Cell: A Molecular Approach by G.M. Cooper, 8th Ed.

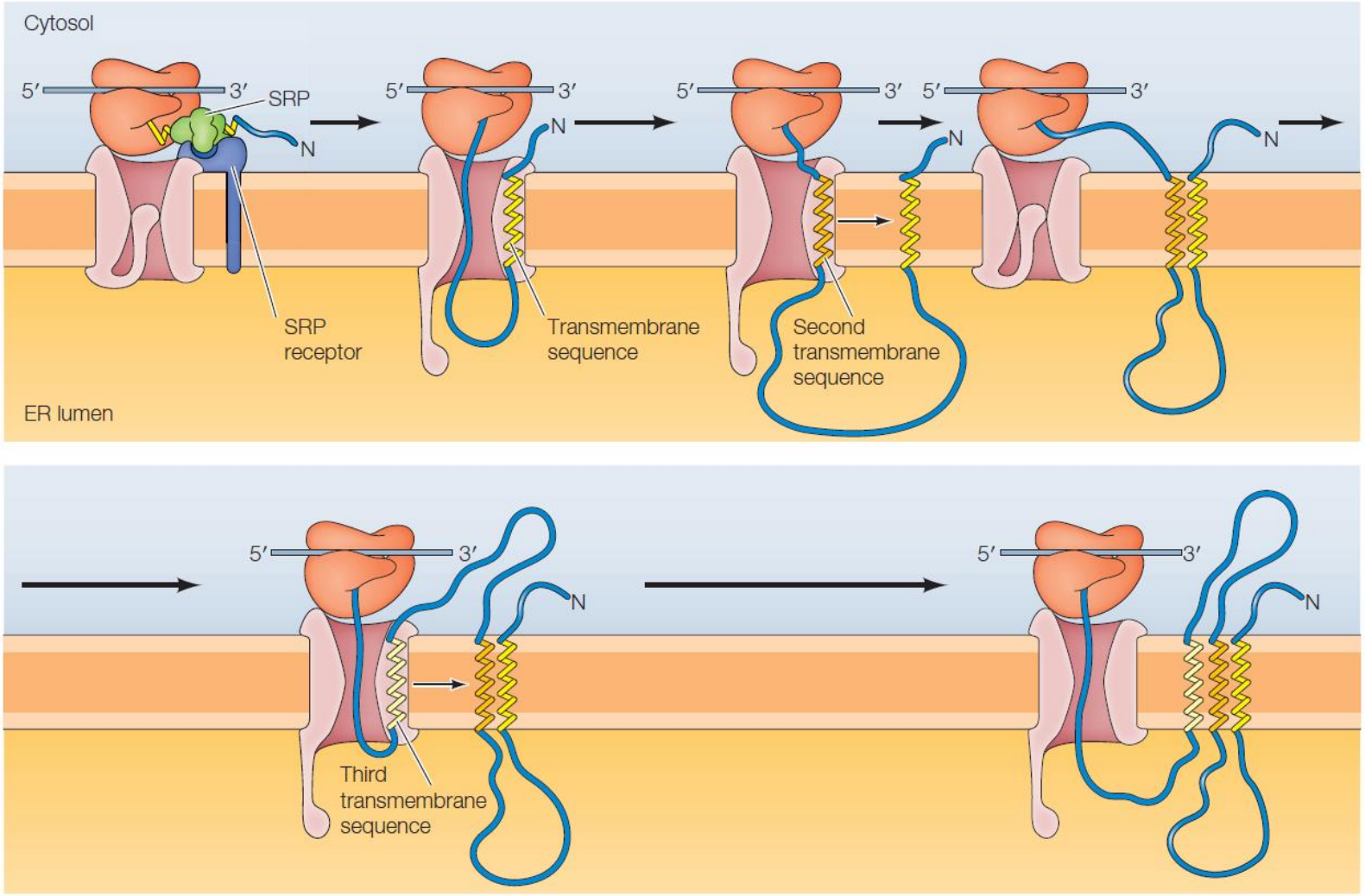
Insertion of membrane proteins via internal transmembrane sequences



Internal transmembrane sequences can lead to the insertion of polypeptide chains in either orientation in the ER membrane. (B) Other internal transmembrane sequences are oriented to direct the transfer of the amino-terminal portion of the polypeptide across the membrane. Continued translation results in a protein that spans the ER membrane with its amino terminus in the lumen and its carboxy (C) terminus in the cytosol

The Cell: A Molecular Approach by G.M. Cooper, 8th Ed.

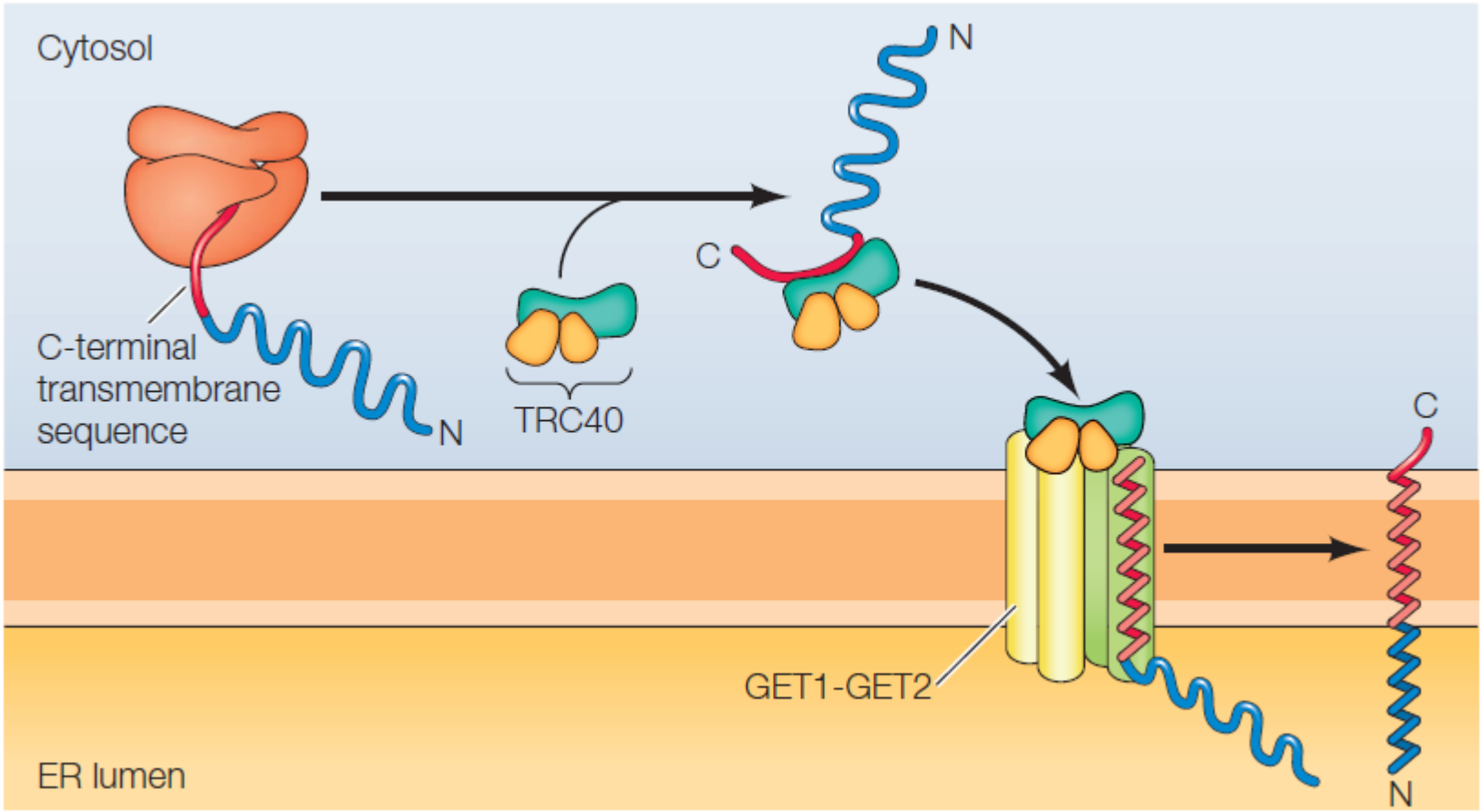
Insertion of a protein that spans the membrane multiple times



In this example, an internal transmembrane sequence results in insertion of the polypeptide chain with its amino (N) terminus on the cytosolic side of the membrane. Translation proceeds until a second transmembrane sequence is encountered. This causes the polypeptide chain to form a loop within the lumen of the ER; translation continues in the cytosol. A third transmembrane sequence reopens the channel, triggering reinsertion of the polypeptide chain into the translocon and forming a loop in the cytosol. The process can be repeated many times, resulting in the insertion of proteins with multiple membrane-spanning regions.

The Cell: A Molecular Approach by G.M. Cooper, 8th Ed.

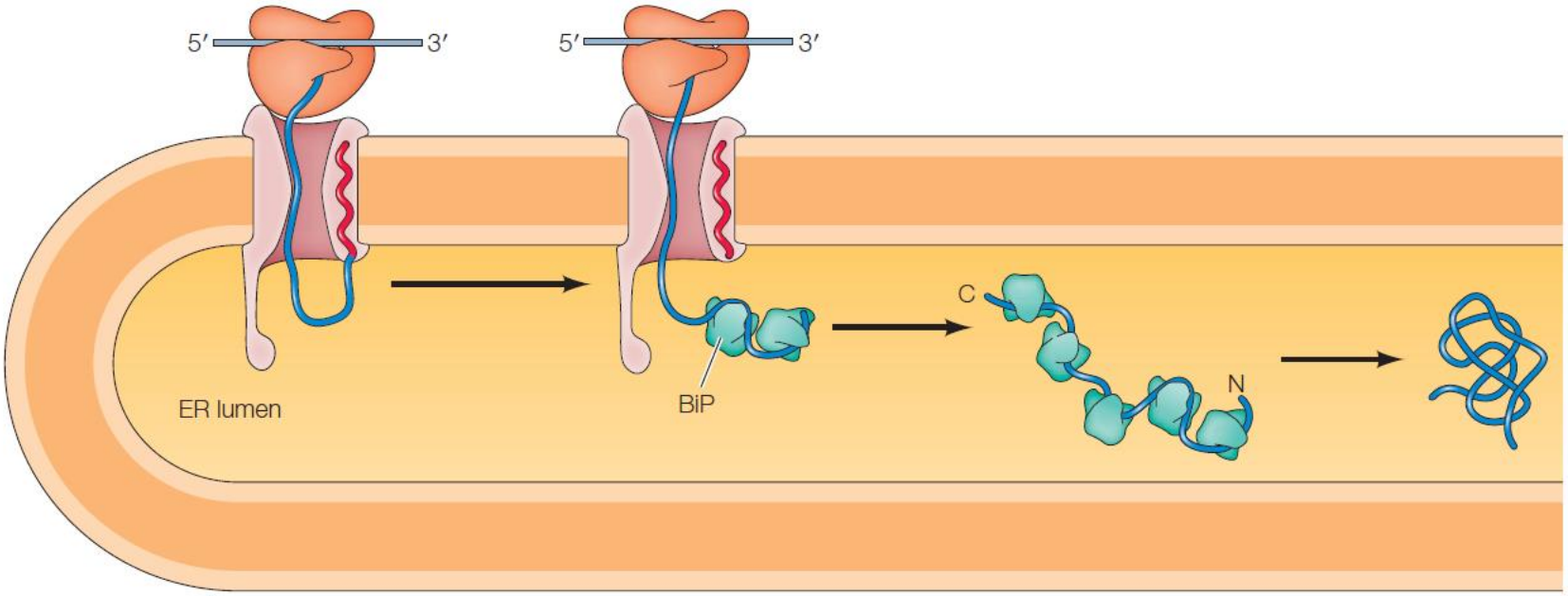
Posttranslation insertion of a protein with a C-terminal transmembrane sequence



Proteins with a transmembrane sequence at their carboxy (C) terminus are not recognized by SRP because the C-terminal sequence does not exit the ribosome until translation is complete. Instead, these proteins are recognized posttranslationally by the targeting factor TRC40, which brings them to the GET1-GET2 receptor. They are inserted into the ER membrane with their short C-terminal domains on the cytosolic side

The Cell: A Molecular Approach by G.M. Cooper, 8th Ed.

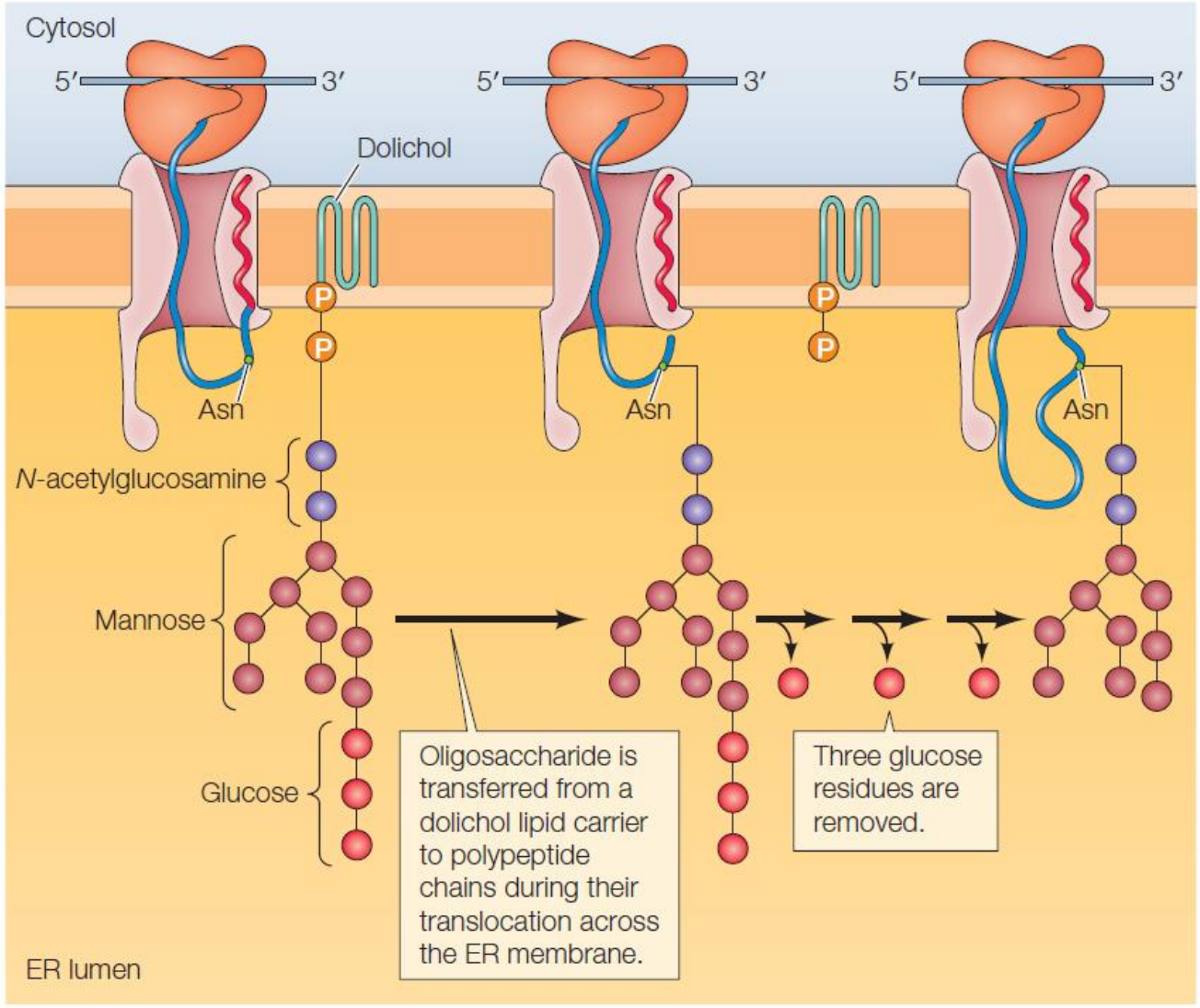
Protein folding in the ER



The molecular chaperone BiP binds to polypeptide chains as they cross the ER membrane and facilitates protein folding within the ER.

The Cell: A Molecular Approach by G.M. Cooper, 8th Ed.

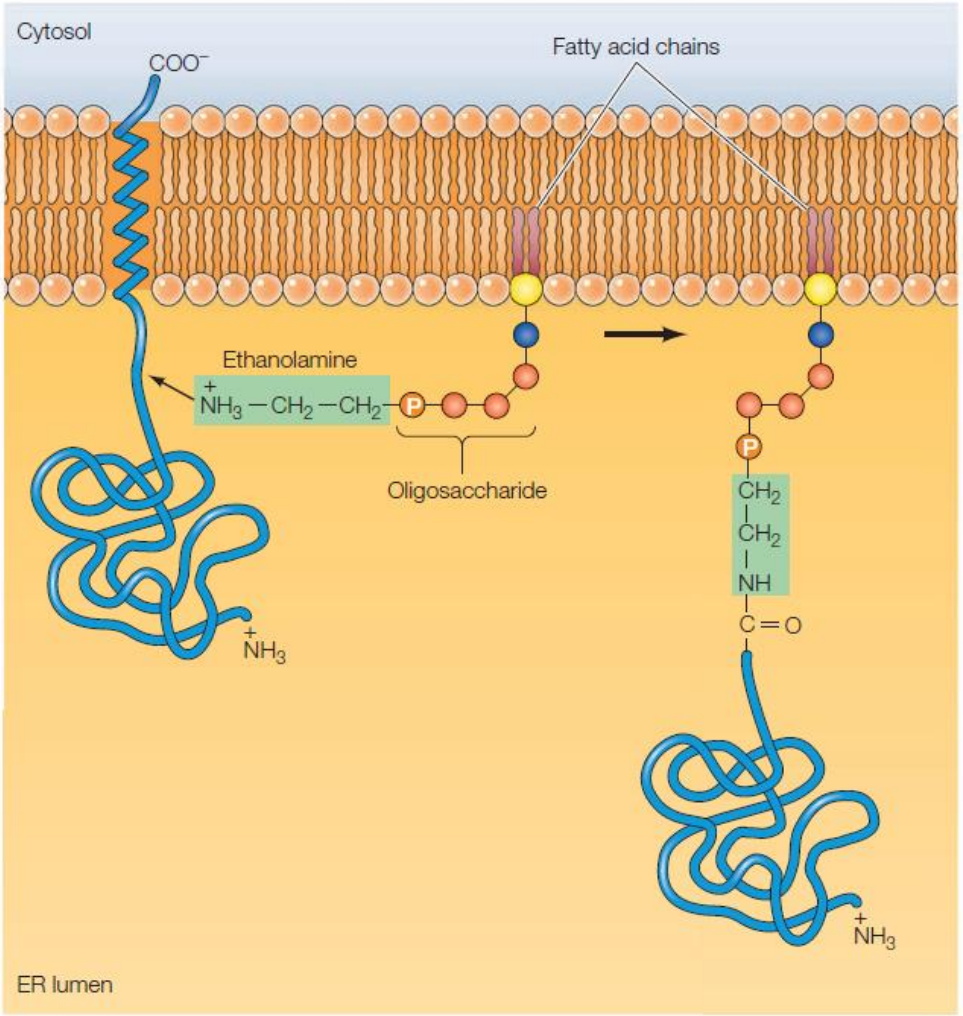
Protein glycosylation in the ER



Proteins are glycosylated within the ER by the addition of a 14-sugar oligosaccharide to an acceptor asparargine (Asn) residue. Three glucose residues are removed while the protein is still within the ER

The Cell: A Molecular Approach by G.M. Cooper, 8th Ed.

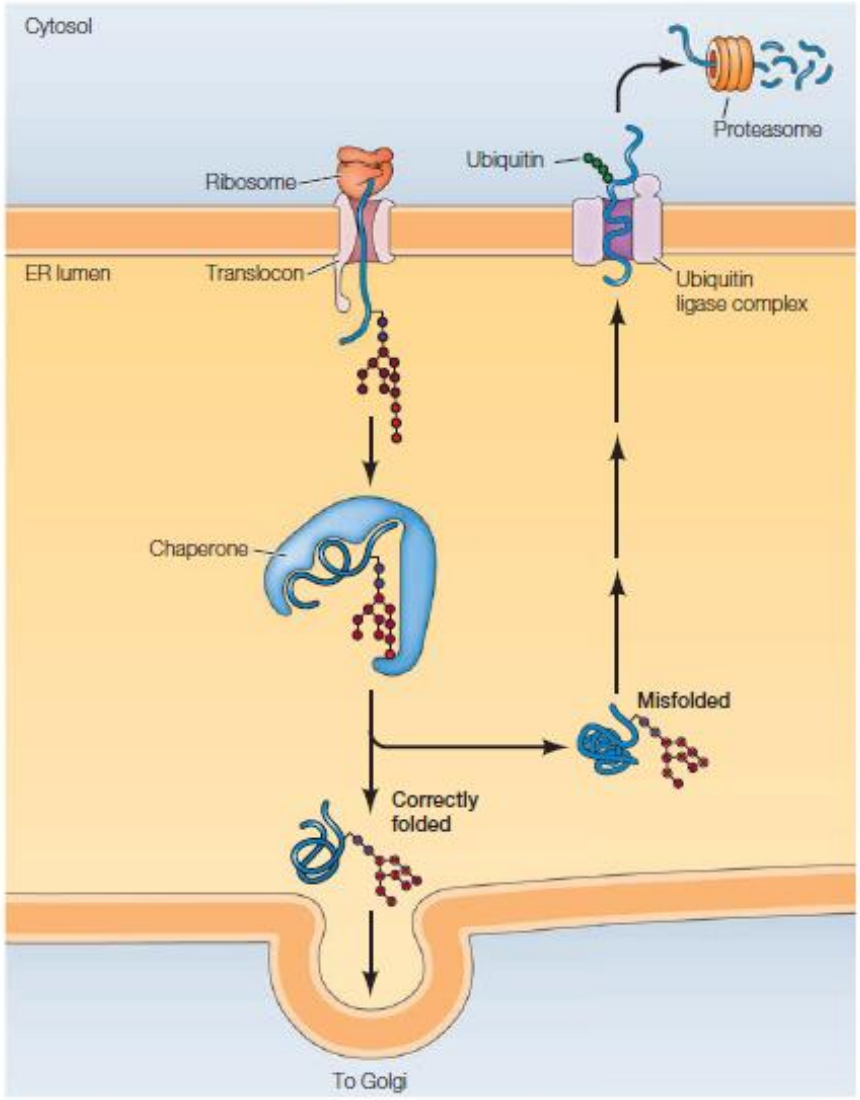
Addition of GPI anchors



GPI anchors are assembled in the ER and added to polypeptides anchored in the membrane by a carboxy-terminal membrane-spanning region. The membrane-spanning region is cleaved, and the new carboxy terminus is joined to the NH2 group of ethanolamine, leaving the protein attached to the membrane by the GPI anchor, which contains two fatty acid chains linked to an inositol head group and an oligosaccharide portion consisting of mannose and glucosamine residues

The Cell: A Molecular Approach by G.M. Cooper, 8th Ed.

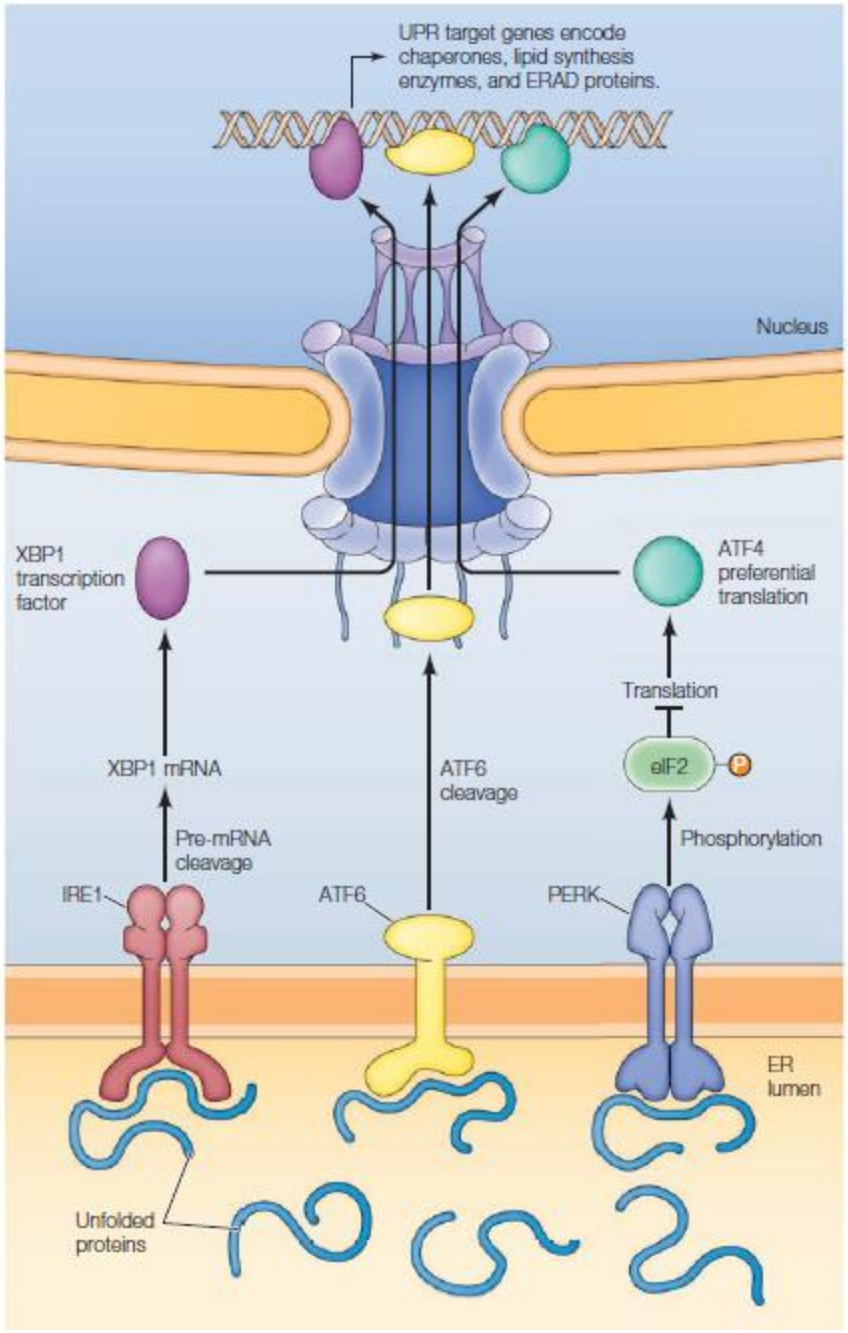
Glycoprotein folding



As the glycoprotein exits the translocon, chaperones bind and assist in folding. If the protein is correctly folded it proceeds to exit the ER. However, if too many hydrophobic regions are exposed, indicating improper folding, the protein is targeted back to the cytosol through a ubiquitin ligase complex in the ER membrane. The protein is ubiquitylated at the cytosolic side of this complex and degraded in the proteasome.

The Cell: A Molecular Approach by G.M. Cooper, 8th Ed.

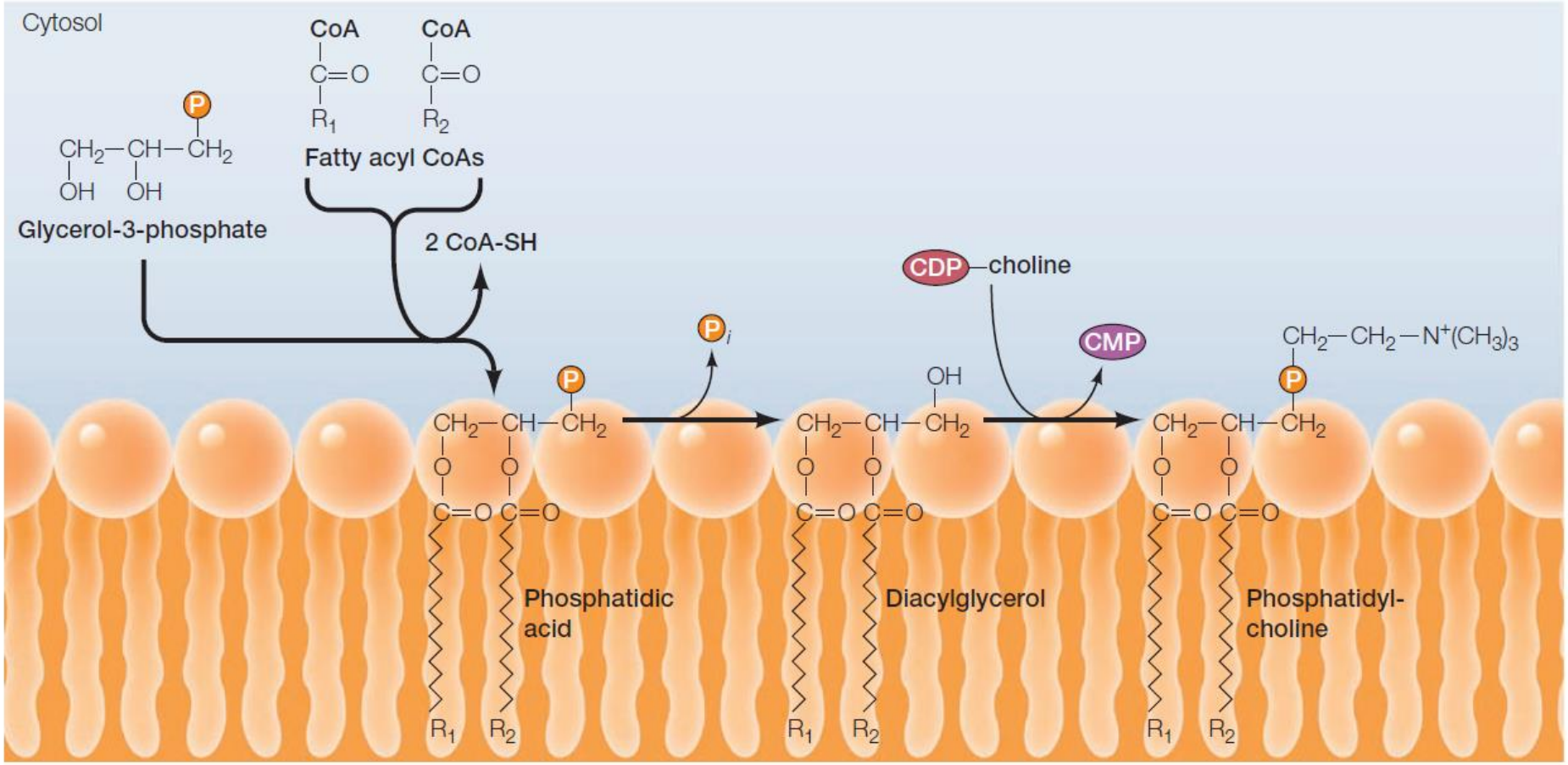
Unfolded protein response (UPR)



Unfolded proteins activate three receptors in the ER membrane. The first, IRE1, cleaves premRNA of a transcription factor (XBP1). This leads to synthesis of XBP1, which translocates to the nucleus and stimulates transcription of UPR target genes. The second receptor, ATF6, is cleaved to release the active ATF6 transcription factor. The third receptor, PERK, is a protein kinase that phosphorylates the translation factor eIF2. This inhibits general translation, reducing the amount of protein entering the ER. It also results in preferential translation of the transcription factor ATF4, which further contributes to the induction of UPR target genes encoding chaperones, enzymes involved in lipid synthesis, and ERAD proteins.

The Cell: A Molecular Approach by G.M. Cooper, 8th Ed.

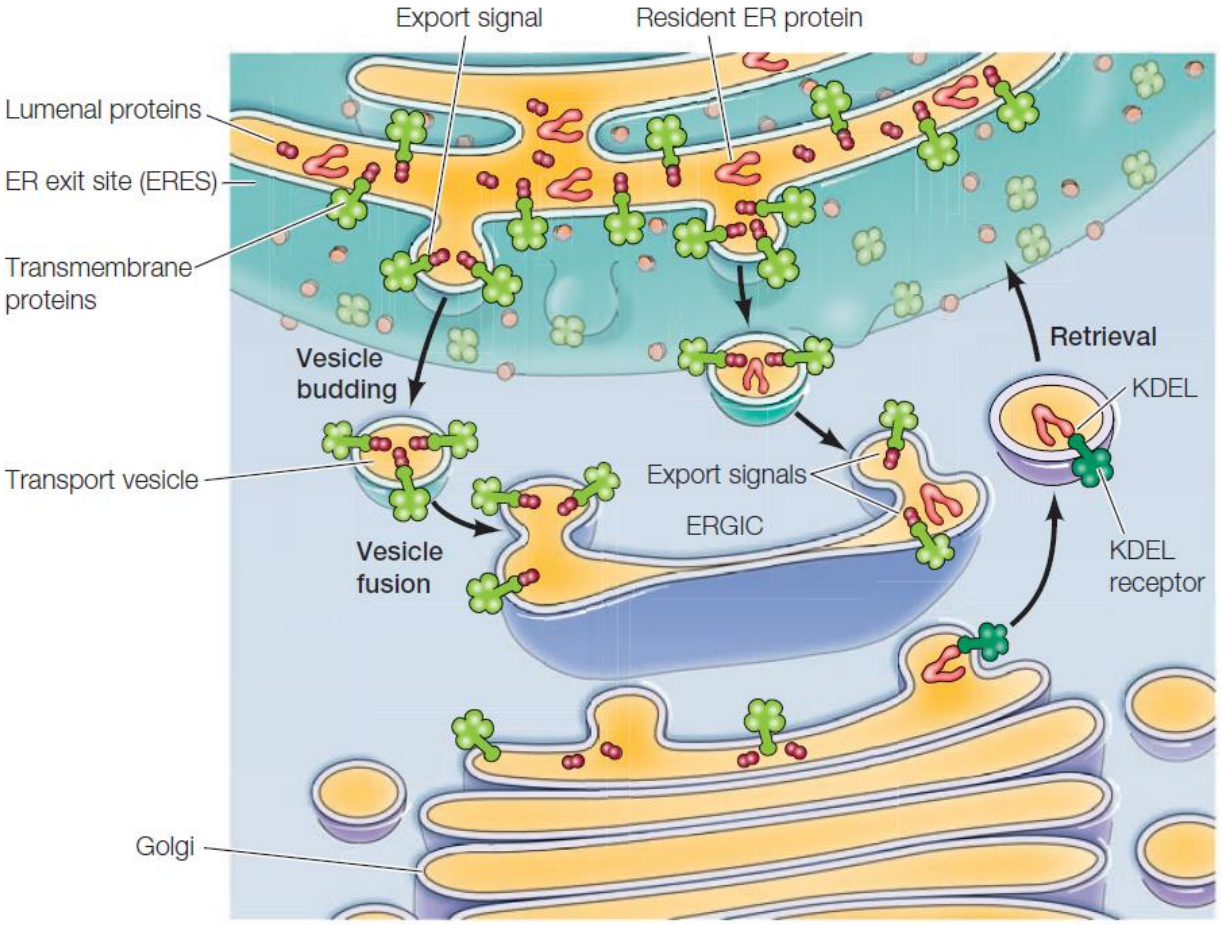
Synthesis of a phospholipid



Glycerol phospholipids are synthesized in the ER membrane from cytosolic precursors. Two fatty acids linked to coenzyme A (CoA) carriers are first joined to glycerol-3-phosphate, yielding phosphatidic acid, which is simultaneously inserted into the membrane. A phosphatase then converts phosphatidic acid to diacylglycerol, which is converted to phosphatidylcholine by addition of a polar phosphocholine head group

The Cell: A Molecular Approach by G.M. Cooper, 8th Ed.

Vesicular transport from the ER to the Golgi



Proteins and lipids are carried from the ER to the Golgi in transport vesicles that bud from ER exit sites (ERES), fuse to form the vesicles and tubules of the ER–Golgi intermediate compartment (ERGIC), and are then carried to the Golgi. Lumenal ER proteins targeted for the Golgi are bound by transmembrane proteins that are selectively packaged into vesicles. Resident ER proteins destined to remain in the lumen of the ER are marked by KDEL retrieval sequences at their carboxy terminus. If these proteins are exported from the ER to the Golgi, they are recognized by a recycling receptor in the ERGIC or the Golgi and selectively returned to the ER.

The Cell: A Molecular Approach by G.M. Cooper, 8th Ed.

Thank You